Remarks

Claims 1, 6, 7, 10, and 14-21 are pending. Claim 17 has been amended to correct antecedent basis and to recite "3-ketohexanoyl CoA". Other amendments are discussed below.

Rejection Under 35 U.S.C. § 112, written description

Claims 1, 6, 7, 10, and 14-21 were rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor had possession of the claimed invention. Applicants respectfully traverse this rejection as applied to the amended claims.

Support for claim 1 can be found, for example, in Example 3, which describes the production of PHBH by construction of transgenic *E. coli* strains that express chromosomally encoded phaC from *N. salmonicolor*. Note that the only polymerase that is provided is the one obtained from *N. salmonicolor*, which acts on both substrates. As demonstrated by Example 5, the polymerase from *A. caviae* can also form a PHBH copolymer. Accordingly the specification discloses enzymes that accept both 3-HB and 3-HH as substrates.

Other sources for suitable enzymes are also available. At the end of Example 1, on page 21, lines 11-15, the specification states, "Alternative 3-hydroxyhexanoyl CoA accepting PHA polymerase genes (i.e other than phaC from *N. salmonicolor*) can be obtained from organisms that have been shown to incorporate this monomer, including *A. caviae, C. testosteroni, T. pfenigii,* and possibly *P. denitrificans* and *S. natans*. These genes can be expressed in *E. coli* according to the same procedures described above." Example 5 demonstrates that this statement is not mere conjecture but substantiated.

Rejection Under 35 U.S.C. § 112, enablement

Claims 1, 6, 7, 10, and 14-21 were rejected under 35 U.S.C. § 112, first paragraph, as not being enabled. Applicants respectfully traverse this rejection.

In response to the statements at page 4 that applicants "does not describe the composition or structure for any of the genes of the generic functional categories", the examiner's attention is drawn to the Background of the Invention, which states as follows:

"Methods for producing PHAs in natural or genetically engineered organisms are described by Steinbüchel, "Polyhydroxyalkanoic Acids" in *Biomaterials* (Byrom, ed.) pp. 123-213 (MacMillan Publishers, London 1991); Williams & Peoples, *CHEMTECH*, 26:38-44 (1996); Steinbüchel & Wiese, *Appl. Microbiol. Biotechnol.*, 37:691-97 (1992); U.S. Patent Nos. 5,245,023; 5,250,430; 5,480,794; 5,512,669; 5,534,432 to Peoples and Sinskey (which also disclose and claim the genes encoding reductase, thiolase, and PHB polymerase); Agostini *et al., Polym. Sci.*, Part A-1, 9:2775-87 (1971); Gross *et al., Macromolecules*, 21:2657-68 (1988); Dubois, *et al., Macromolecules*, 26:4407-12 (1993); Le Borgne & Spassky, *Polymer*, 30:2312-19 (1989); Tanahashi & Doi, *Macromolecules*, 24:5732-33 (1991); Hori *et al., Macromolecules*, 26:4388-90 (1993); Kemnitzer *et al., Macromolecules*, 26:1221-29 (1993); Hori *et al., Macromolecules*, 26:5533-34 (1993); Hocking & Marchessault, Polym. Bull., 30:163-70 (1993); Xie et al., Macromolecules, 30:6997-98 (1997); and U.S. Patent No. 5,563,239 to Hubbs et al."

Should the examiner decide to look at any of these publications, all of which were cited by applicants in their Information Disclosure Statement, it will become immediately apparent

that they have indeed provided numerous sources of enzymes, including the sequences and characterization of the encoded enzymes.

Generally, there is an inverse correlation between the level of skill and knowledge in the art and the specificity of disclosure necessary to satisfy the written description requirement. Information which is well known in the art need not be described in detail in the specification. See e.g. *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1379-80, 231 USPQ 81, 90 (Fed. Cir. 1986). As stated in the Amendment filed on February 3, 2004, Applicants are claiming a method for the production of a polymer in bacteria that incorporates new combinations of genes and enzymes with **known** sequences. The level of skill in the art is high- one of ordinary skill would know that each of the genes and enzymes used in the claimed methods can be easily isolated and sequenced using methods known in the art or described in the specification. In addition sequence information can be obtained from the cited publications, and actual DNA can be obtained from the authors of the cited publications or purchased from commercial suppliers, such as the American Type Culture Collection (ATCC). In addition, published amino acid and nucleotide sequence listings for the various genes can be obtained from GenBank or the National Center for Biotechnology Information, as demonstrated by the Applicants in their response and amendment mailed on March 10, 2003. As a further example, Applicants enclose copies of the results of searches of GenBank for *acyl CoA synthase, acyl ACP thioesterase,* and *ACP-CoA transacylase* sequences published before the priority date of this application, January 22, 1998. This type of a search may be perfonned to obtain the sequence of

any of the claimed genes. Finally, on page 24, line 8-9, the specification makes reference to the Japanese Culture Collection, which is a public depository.

The enzymes set forth in the claims are a phbA thiolase gene, a phbB reductase gene, a phbC polymerase gene that encodes an enzyme that polymerizes 3-hydroxybutyryl CoA and 3-hydroxyhexanoyl-CoA (claim 1), a gene encoding a ß-hydroxyacyl-ACP-coenzyme A transferase (claim 10), a gene encoding a D-specific enoyl-CoA hydratase (claim16), three enzymes from *C. acetobutylicum* that form butyryl CoA, a thiolase specific for 3-ketohexanoyl CoA, a reductase specific for 3-ketohexanoyl CoA (claim 17), and fatty acid biosynthetic enzymes including ACP-CoA transacylase, acyl ACP thioesterase, and acyl CoA synthase (claim 20). Each of these genes and enzymes were well known to those skilled in the art, commercially available and sufficiently identified in the specification as of the date of filing to reasonably convey to one skilled in the art that the inventors had possession of the claimed invention.

As noted above, on page 1, line 29 to page 2, line 11, the specification discloses a number of publications, which describe methods for producing PHAs in natural or genetically engineered organisms and makes special reference to patents that disclose the genes encoding reductase, thiolase, and PHB polymerase (U.S. Patent Nos. 5,245,023; 5,250,430; 5,480,794; 5,512,669; 5,534,432 to Peoples and Sinskey; page 2, lines 2-5). In addition, on page 10, line 29 to page 11, line 2, the specification states that useful PHA synthase genes have been isolated from, for example, *Aeromonas caviae* (Fukui & Doi, *J. Bacteriol.* 179: 4821-30 (1997)), *Rhodospirillum rubrum* (U.S. Patent No. 5,849,894), *Rhodococcus ruber* (Pieper & Steinbuechel, *FEMS*

Microbiol.Lett. 96(1): 73-80 (1992)), and *Nocardia corallina* (Hall et. al., *Can. J. Microbiol.* 44: 687-91 (1998)).

The ß-hydroxyacyl-ACP-coenzyme A transferase gene encodes an enzyme that converts 3-hydroxyacyl ACP to the CoA derivative. This step in the polyhydroxyalkanoate pathway is facilitated by acyl ACP:CoA transferase activity. The specification states that genes that encode this enzyme can easily be identified in bacteria that produce polyhydroxyalkanoates from oxidized carbon sources, such as carbohydrates (see page 15, lines 18-23, of the specification). In addition, the identification of genes encoding enzymes that convert acyl ACP to acyl CoA is presented in Figure 10 as a screen that makes use of the very user friendly *lux* system of *Vibrio fischeri*. One merely assays for light generation that results from the induction of the transgenic lux system. Such light generation implicates ACP::CoA transferase activity that is present in the system.

On page 24, lines 4-9, the specification recites a specific reference relating to the *phaJ* gene encoding an enoyl-CoA hydratase (Fukui and Doi, *J. Bacteriol.* 179: 4821-30 (1997)), and describes how to isolate this gene from chromosomal DNA prepared from *A. caviae* strain FA-440, obtained from the Japanese Culture Collection under accession number FERM BP 3432 (U.S. Patent No. 5,292,860)).

On page 12, line 18 to page 13, line 19, the specification describes a thiolase specific for 3-ketohexanoyl, a reductase specific for 3-ketohexanoyl, and enzymes from *C. acetobutylicum* that form butyryl CoA. With regard to the enzymes from *C. acetobutylicum*, a number of publications are recited, which describe the isolation of these genes. The specification also

makes reference to GenBank (page 13, line 4), demonstrating that the sequences of these genes may be accessed through a public depository. In addition, on page 11, lines 10-18, the specification recites Ploux *et al.* (1988) and Haywod et al.(1988), which disclose that 3-ketohexanoyl CoA is a substrate for reductase enzymes from *Z. ramigera* and *R. eutropha*. The specification also recites Haywood *et al.* (1988) on page 11, lines 19-24, which discloses that *R. eutropha* has two 3-ketothiolases, one of which has activity for higher 3-ketoacyl CoA's (i.e. 3-ketohexanoyl CoA).

Finally, the fatty acid biosynthetic enzymes are defined by their substrates. Many are known, cloned and well characterized. For example, see the enclosed copies of the results of searches of GenBank for *acyl CoA synthase*, *acyl ACP thioesterase*, and *ACP-CoA transacylase*. Homologous genes are readily isolated from bacteria such as *R. eutropha*, *A. latis*, *C. testosteroni*, *P. denitrificans*, *R. ruber*, and other PHA and non-PHA producers using the same methods to identify the *fao*AB (fatty acid oxidation) genes in *P. putida* KT2442. This is explicitly stated at lines 30-3, bridging pages 14 and 15 of the specification. Furthermore, epimerase activity had been detected in the fatty acid oxidation complexes of *E. coli* and *P. fragi*. As disclosed at page 14, lines 21-26, each of the *Fao*AB complex subunits were cloned and expressed to show the substrate specificity of components of the PHA pathway in *P. putida*.

A proper analysis of the *Wands* factors shows that the claims satisfy the enablement requirement. The courts have indicated that some experimentation is permitted as long as such experimentation is not undue. As stated in *MIT v. A.B. Fortia*, "The fact that experimentation may be complex does not make it undue if the art typically engages in such experimentation".

It clear from the amount of direction or guidance presented in the specification, the presence of working examples, the state of the prior art, and the relative skill in the art that one of ordinary skill in the art would be able to make and use the claimed transgenic bacteria for the production of polyhydroxybutyrate-co-polyhydroxyhexanoate without **undue experimentation.**

As discussed above, the specification clearly discloses how to obtain the genes and enzymes that are used in the methods and recites specific publications which describe these materials in detail. For example, on page 1, line 29 to page 2, line 11, the specification discloses a number of publications, which describe methods for producing PHAs in natural or genetically engineered organisms. In addition, on page 10, line 29 to page 11, line 2, the specification discloses a number of organisms from which useful PHA synthase genes have been isolated. As described above, there is adequate support in the specification for all of the claimed genes and enzymes. Furthermore, the genes and enzymes may be isolated using methods commonly known in the art or described in the publications, obtained from the authors of the cited publications, or purchased from commercial suppliers, such as the American Type Culture Collection (ATCC).

Procedures used to isolate genetic sequences are well known and can be applied to the isolation of any of the genes used in the claimed methods. Although there is no requirement for examples, Applicants have provided numerous working examples which not only demonstrate that one can use the claimed enzymes to produce HH containing copolymers, but that one can isolate the desired enzymes with only routine experimentation. Example 1, on page 19 of the specification, discusses a routine method used for the isolation of specific genes. This Example

illustrates the amplification and isolation of the *pha*C gene, encoding the polyhydroxyalkanoate ("PHA") polymerase enzyme, from *N. salmonicolor* chromosomal DNA. Example 3 further shows, using the same methods of Example 1, how one would isolate the *hbd*, *crt*, and *bdh* genes from *C. acetobutylicum* (see page 22 of the specification).

Once a gene is identified, it is routine in the art to incorporate the gene into a plasmid for transfection of bacteria. There is sufficient direction and guidance given by the specification to construct plasmids and express the claimed genes in bacteria (see page 18, lines 15-28 and Examples). Furthermore, the experimental protocols are routine in the art and expression vectors, restriction enzymes and ligation enzymes are also commerically available.

The enzymes are defined by their substrate specificity. As discussed at page 5, lines 25-27, of the specification, "the genes are preferably selected on the basis of the substrate specificity of their encoded enzymes being beneficial for the production of the 3HH polymers." The substrate, in the presence of its cognate active enzyme, will be readily converted into product (i.e. the substrate for another enzyme). Based upon the specification, one of ordinary skill in the art will appreciate that the presence, or production, of end-product (i.e. polyhydroxybutyrate-co-polyhydroxyhexanoate) is easily measured and characterized using methods well known in the art.

Applicants are not claiming the enzymes or their genes alone; they are claiming a system for making polymers by bacterial fermentation wherein the useful substrates, and resulting products, are modified by the selection of the genes expressed by the host expression system,

using **known** genes and enzymes, which can be easily expressed in bacteria through known methods in the art.

Applicants have patents on engineering of bacteria and plants to express a phbC polymerase gene, phbA thiolase gene and a phbB reductase gene issued on patent applications filed in 1987, more than fifteen years ago. Applicants have spent those fifteen years identifying other enzymes that can be used to modify the resulting polymers. The massive amount of prior art clearly demonstrates that the field is not unpredictable, and that once one identifies the enzymes to be used, based on their known substrates and known reaction products, it becomes routine to express the genes encoding those enzymes.

Rejection Under 35 U.S.C. § 112, second paragraph

Claim 17 was rejected under 35 U.S.C. § 112, second paragraph, as being indefinite. Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

Claim 17 has been amended to correct antecedent basis.

Allowance of claims 1, 6, 7, 10, and 14-21, as amended, is respectfully solicited.

Respectfully submitted,

Rivka D. Monheit

Rivka D. Monheit
Reg. No. 48,731

Date: August 5, 2004

PABST PATENT GROUP, LLP
400 Colony Square, Suite 1200
1201 Peachtree Street
Atlanta, Georgia 30361
(404) 879-2151
(404) 879-2160 (fax)